British Journal of Venereal Diseases, 1979, **55**, 348-350

Vaginal oxytetracycline concentrations

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SUMMARY Although tetracycline preparations are widely used in departments of genitourinary medicine, or sexually transmitted diseases clinics, little is known of the concentrations of these preparations in genital secretions. For this reason a microbiological method was used for estimating oxytetracycline concentrations in vaginal secretions. These concentrations varied from 0·6 to 6·5 μg/ml in 19 women who had had sexual contact with a man with non-specific urethritis and who were taking oxytetracycline dihydrate 250 mg four times daily. They were well in excess of the minimum inhibitory concentration of oxytetracycline (0·2 μg/ml) for the strains of *Chlamydia trachomatis* isolated from the patients with positive culture results. Thus, oxytetracycline 250 mg four times daily appears to be a satisfactory regimen for the treatment of chlamydial genital infection in women.

Introduction

The commonest condition diagnosed among men attending departments of genitourinary medicine or sexually transmitted diseases (STD) clinics in England is non-specific or nongonococcal urethritis (NSU) (Department of Health and Social Security, 1977).

There is increasing evidence that in many cases NSU is due to *Chlamydia trachomatis* (Richmond and Oriel, 1978; Schachter, 1978). *In-vitro* studies show that this agent is susceptible to oxytetracycline (Treharne *et al.*, 1977; Ridgway *et al.*, 1978). Even where this organism is not isolated, patients are usually treated with a tetracycline compound (King and Nicol, 1975a; Morton, 1975), so large amounts of tetracycline are prescribed in STD clinics. Furthermore, non-specific genital infection (NSGI) is frequently diagnosed in women either on the basis of chlamydial isolation (Alani *et al.*, 1977) or because of contact with a man with NSU (King and Nicol, 1975b), and the number of such cases is also increasing (Department of Health and Social Security, 1977); many of these women are also treated with tetracycline. As far as we can determine, there is remarkably little information on concentrations of antibiotics in genital secretions, so we considered it worthwhile to estimate oxytetracycline concentrations in the genital secretions of women who were contacts of men with NSU.

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Received for publication 23 April 1979

Patients and methods

Twenty women were studied, and at their first attendance general and genital examinations and investigations were carried out as described elsewhere (Burns *et al.*, 1975).

Oral oxytetracycline dihydrate (ICI) 250 mg four times daily for 10 days was prescribed; after the patients had been taking this regimen for seven days they were examined, and vaginal washings were collected using a modification of the method described by Stamey and Condy (1975). A Cusco bivalve speculum was passed, and the vagina was washed out with four 10-ml aliquots of distilled water. The speculum was then removed and rinsed with a further 10-ml aliquot of distilled water, thus giving a total of 50-ml of washings. A 10-ml sample of venous blood was then collected.

Samples were only collected five days after the end of the last menstrual period, and no sample was taken if there was cervical bleeding from any cause. As oxytetracycline produces a plateau, rather than a peak, in the serum concentration two to four hours after ingestion, samples were collected two to four hours after a tablet had been taken.

MICROBIOLOGICAL ASSAY OF VAGINAL OXYTETRACYCLINE CONCENTRATIONS

Preparation of vaginal secretion

The vaginal washout liquid was freeze-dried in an Edwards high-vacuum freeze-drier. The dried

specimen was then reconstituted with 5 ml of distilled water, yielding a semi-homogeneous suspension, which was used for the antibiotic assay.

Determination of dilution factor

The dilution factor of the reconstituted secretion was measured using the method described by Stamey and Condy (1975). This is based on measurement of sodium ion concentration [Na^+] in the undiluted and in the reconstituted secretion. The ratio of [Na^+] in the undiluted vaginal secretion to that in the reconstituted secretion represented the dilution factor.

Twenty microlitres of secretion were collected directly from the vagina of 10 patients using a Finpipette and added to 980 µl of deionised water. The vaginal washout in 50 ml distilled water was then carried out and freeze-dried as described above. Twenty microlitres of the reconstituted suspension of the vaginal washout were treated in the same manner as the pure secretion. One millilitre 10% (w/v in deionised water) trichloracetic acid (protein precipitating grade BDH) was added to both samples, the mixtures were vortexed and centrifuged at 3000 r/min. for five minutes, and the [Na^+] was estimated in the clear, supernatant liquids using a flame photometer (Evans Electroselenium Limited).

ASSAY METHOD

Microbiological assay was carried out to estimate concentrations of oxytetracycline in serum and reconstituted vaginal secretion using DST agar (Oxoid) and *Staphylococcus aureus* NCTC 6571 as test organism. Square plates (23 × 23 cm) were used and 130 ml molten agar (pH 6·8) was poured into each plate; after solidification, the surface was irrigated with 20 ml of 1/100 dilution of an overnight growth of the test organism (approximately 10^7 micro-organisms/ml), the excess liquid was removed, and the plate was dried for 10 minutes at 37°C. Twenty-five holes were punched in the agar and filled with equal quantities of the specimens and standard solutions of oxytetracycline dihydrate in water and serum in concentrations of 0·125, 0·25, 0·5, 1·0, 2·0, 3·0, 4·0, 5·0, 7·0, 8·0, and 10·0µg/ml. The plate was then incubated for 18 hours at 37°C and the diameter of each zone of inhibition measured using a zone reader (Leebrook Scientific and Electronic Eng. Co. Ltd), and the corresponding concentrations were calculated from a standard curve. The concentration of the antibiotic in the reconstituted secretion was multiplied by the dilution factor to determine the concentration in pure secretion.

C. TRACHOMATIS STUDIES

C. trachomatis was cultured on irradiated McCoy cells using the method described by Darougar *et al.* (1971). The minimum inhibitory concentration (MIC) of oxytetracycline was determined as described by Treharne *et al.* (1977).

Results

The dilution factor of the pure secretion/reconstituted secretion in 10 pairs of samples varied from 3·1 to 6·4 (Table 1) with a standard deviation of 1·02 and coefficient of variation of 20·54; the mean was 4·96, which is similar to the value of 5·0 obtained by Stamey and Condy (1975).

Twenty patients were studied, whose ages ranged from 18 to 39 years with a mean of 25·2 years. All were white; 14 were single and six were married. Each was a sexual contact of a man with NSU and represented all such women who attended seven days after starting therapy during the period of the study and who fulfilled the criteria outlined. The results of the microbiological assay of oxytetracycline in the

Table 1 *Determination of the dilution factor of vaginal secretions*

Age (years)	[Na^+] reading*		Dilution factor (Pure/reconstituted)
	Pure secretion	Reconstituted secretion	
33	95	19	5·00
43	82	16	5·13
26	87	19	4·58
26	93	30	3·10
21	75	13	5·77
31	90	25	3·60
19	91	19	4·79
21	77	12	6·42
18	84	16	5·25
26	89	15	5·93

*Flame photometer readings

Table 2 *Results of microbiological assay of oxytetracycline dihydrate in vaginal secretion and in serum*

Case no.	Age (years)	Oxytetracycline concentration (µg/ml)		
		Pure vaginal secretion	Serum	Ratio*
1	25	0·63	1·70	0·37
2	28	2·00	3·50	0·57
3	26	0·60	2·30	0·26
4	23	1·00	2·70	0·37
5	25	1·25	0·20	6·25
6	23	0·00	2·90	
7	20	1·00	2·25	0·45
8	39	0·60	NS	
9	18	0·60	1·30	0·46
10	29	0·60	2·30	0·26
11	22	0·60	2·00	0·30
12	30	0·60	0·70	0·86
13	21	6·50	3·00	2·17
14	19	4·50	2·20	2·05
15	18	0·60	3·70	0·16
16	32	4·50	3·70	1·22
17	37	3·50	2·50	1·40
18	19	0·63	2·50	0·25
19	23	4·00	3·40	1·18
20	27	2·00	2·70	0·74

*Ratio = vaginal secretion: serum
NS = no blood specimen collected

samples of vaginal secretion and serum are given in Table 2. Oxytetracycline concentrations in vaginal secretion ranged from 0 to 6·5 μg/ml and in serum from 0·2 to 3·7 μg/ml.

C. TRACHOMATIS CULTURES

Specimens for culture of *C. trachomatis* were taken from the cervical os of 17 women; no result was obtained from one specimen because of poor cell growth; six (31·2%) of the remaining cultures gave a positive result. The MICs of oxytetracycline were 0·2 μg/ml for two cultures with positive results from one patient, 0·1-0·2 μg/ml for one from a second patient, and 0·2 μg/ml for one from a third patient. Unfortunately, estimates could not be made on the other two cultures with positive results for technical reasons.

Discussion

C. trachomatis is now regarded as having an important role in the pathogenesis of NSU and NSGI (Richmond and Oriel, 1978). This organism can be cultured from up to half of unselected cases of NSU in men and from 30-35% of women who have had sexual contact with men with NSU (Richmond and Oriel), 1978). The presence or absence of chlamydiae in these women may be significantly associated with its isolation from their contacts (Alani *et al.*, 1977).

The MIC of oxytetracycline for *C. trachomatis* has been reported to vary from 0·03 μg/ml to 0·5 μg/ml for laboratory strains and clinical isolates from patients in London (Treharne *et al.*, 1977; Ridgway *et al.*, 1978). The MIC results (0·1-0·2 μg/ml) obtained in the present study are within this range and with the exception of one patient, the vaginal concentrations of 0·6-6·5 μg/ml exceeded the minimum inhibitory concentrations. All vaginal concentrations exceeded the maximum recorded MIC of 0·5 μg/ml (Treharne *et al.*, 1977).

The exception, Case 6, with a vaginal concentration of zero, had a serum concentration above the mean, and the zero concentration obtained in vaginal secretion may have been due to a procedural error. Apart from this one case, it is apparent that after treatment with oxytetracycline 250 mg four times daily for seven days adequate concentrations are produced in the vaginal secretion to inhibit *C. trachomatis*. In these cases the vaginal oxytetracycline concentrations were related to the MICs for chlamydiae. Treatment with tetracycline, however, is also important in men with chlamydia-negative NSU and in their female contacts (Thambar *et al.*, 1979).

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